# EFFECT OF THE GROWTH RATE ON THE LEVEL OF THE DNA-DEPENDENT RNA POLYMERASES IN *SACCHAROMYCES CEREVISIAE*

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## 1. Introduction

The presence of multiple RNA polymerases in eukaryotic organisms [1–3] suggests that they may play different roles in the control of the RNA synthesis. One approach to study this possible role is to investigate these enzymes in cells grown under conditions which affect the RNA content of the cells, such as the growth rate.

Our results indicate that in the yeast *Saccharomyces cerevisiae* there is a relationship between the growth rate, the RNA content of the cells and the level of the RNA polymerase I activity, suggesting that the concentration of this enzyme may play a role in the control of the synthesis of the ribosomal RNA and that RNA polymerases I and II are independently regulated.

## 2. Materials and methods

Yeast extract and bactopeptone were obtained from Difco. Nucleotide triphosphates, dithioerythitol (DDT), phenylmethylsulfoxyl fluoride (PMSF) and EDTA from Sigma Chemical Company. 2-Mercaptoethanol from Eastman Kodak Company and DEAE Sephadex A25 from Pharmacia; poly dAT from Biopolymers Inc. and tritium labelled UTP (specific activity 15 Ci/mM) from Schwartz/Mann. The buffer B contains 50 mM Tris–HCl, 5 mM 2-mercaptoethanol, 0.2 mM EDTA and 20% glycerol, pH 7.5.

Two strains of *Saccharomyces cerevisiae*, IL46 and Y185, were used. The growth medium used in the chemostat contained 1% yeast extract 1% peptone, 2% glucose, 0.5% ammonium sulfate, 0.2% ammonium phosphate (dibasic) and 0.1% magnesium sulfate.

Continuous cultivation of the yeast was carried out in a 7 ℓ glass fermentor (Chemap, Inc.). The specific growth rates ($\mu$) were controlled at the desired value by adjusting the flow rate of fresh medium. Oxygen was monitored continuously in the cultures and it was regulated near saturation level. The culture was stirred at the rate of 1200 rpm. The pH was regulated at 5.0 by the automatic addition of NaOH. *S. cerevisiae* IL46 or Y185 was grown at 30°C and samples were withdrawn from the chemostat cultures when growing under steady-state conditions (5–10 generations) at the following specific growth rates: 0.11, 0.15, 0.25, 0.28, 0.35 and 0.43 $hr^{-1}$. These growth rates correspond to generation times of 6.30, 4.60, 2.76, 2.46, 1.97 and 1.6 hr.

The cells from the different samples were suspended in 2 vol of buffer B without glycerol containing ammonium sulfate to a final concn. of 0.5 M. The frozen cell suspension was passed through an Eaton Press under a pressure of 5000 p.s.i. In this condition the breakage of the cells is approx. 90% and the efficiency of the breakage is independent of the conditions of growth of the cells. Glycerol (20%) and PMSF (0.1 mM) were added to the cell homogenate. This cell homogenate was then sonicated in a Branson sonifier for 1 min at the intensity of 3 A and then centrifuged at 50 000 rpm in a SW65 rotor for 1 hr. The clear supernatant (crude extract) was collected and after dialysis against buffer B containing 0.1 mM PMSF, a sample of

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of 15–25 mg protein was subject to chromatography on a 20 ml DEAE Sephadex column to separate the RNA polymerase I and II, which are eluted with 0.25 and 0.3 ammonium chloride in buffer B, respectively [3].

The RNA polymerase activity was assayed in a mixture containing in a volume of 0.25 ml: 50 mM Tris–HCl pH 7.5, 1 mM DTT, 10 mM $MgCl_2$, 2 mM $MgCl_2$, 0.2 mM of each ATP, GTP, CTP, 0.01 mM UTP, 0.5 $\mu$Ci of [$^3$H] UTP (15 Ci/mM), 1 $\mu$g poly dAT as template and 50 $\mu$l of the enzyme solution. The assay mixture was incubated at 30°C for 15 min and the reaction stopped with 2 ml of cold 5% TCA and 1% $Na_6P_2O_7$. The TCA insoluble material was collected on millipore filters, washed and the radioactivity in the dry filters counted in PPO–POPOP cocktail in a Beckman LS-225 liquid scintillation counter. One unit of enzyme activity represents the incorporation of 1 pmol of UMP into TCA insoluble material in 15 min. The RNA content of the cells was obtained from the absorbancy at 260 nm of the total nucleic acids extracted from the cells by the method of Ogur and Rosen [4] after correction for the deoxyribonucleotides present as measured by the diphenylamine reaction following the method of Burton [5]. Proteins were determined by the method of Lowry et al. [6] using bovine serum albumin as standard.

## 3. Results and discussion

The effect of the growth rate on the total amount of RNA per cell in *S. cerevisiae* IL46 is shown in fig. 1. The difference in RNA content in cells growing at growth rates of 0.43 (1.6 hr doubling time) and 0.11 (6.3 hr doubling time) is 3-fold. Over the range studied there is a linear relationship between specific growth rate and RNA content per cell. Since 85% of the total yeast RNA is ribosomal RNA (rRNA) [7], the variations in the cellular RNA content observed are mainly due to variations in the amount of rRNA. The control mechanisms involved in the regulation of the RNA synthesis at different growth rates are as yet unknown. Schweizer and Halvorson [8] ruled out the possibility that the different synthesis of rRNA at various growth rates was due to differential amplification of the ribosomal DNA cistrons. Other possible mechanisms may include template controls involving specific transcriptional "switch off" of some of the 140 ribosomal cistrons present in *S. cerevisiae* or controls at the level of the concentration of the substrates for the RNA polymerase. A reduction in the nucleotide triphosphate pools due to a general metabolic "slow down" in cells growing at low growth rates could produce substrate limiting conditions for the polymerases and in consequence a reduced rate of RNA synthesis.

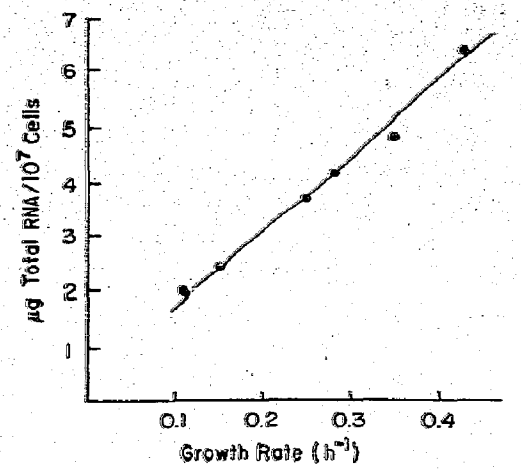


Fig. 1. Total RNA in *S. cerevisiae* IL46 grown at different growth rates. Samples from yeasts grown at different rates under steady-state conditions in the chemostat were collected and total nucleic acids were extracted from $10^{10}$ cells with 1 M perchloric acid at 70°C for 20 min. The extracts were centrifuged and the RNA content was calculated from the absorbancy at 260 nm of the supernatants after correction for the deoxyribonucleosides present which were measured by the diphenylamine reagent according to the method of Burton.

A third possibility for regulation of the RNA synthesis at different growth rates is at the level of the RNA polymerase. To investigate the effect of the growth rate on the RNA polymerases, cells were grown under steady-state conditions in a chemostat and samples corresponding to six specific growth rates were collected. The RNA polymerases were solubilized as described in Materials and methods and the crude extracts were subject to chromatography on 20 ml DEAE Sephadex columns to have a quantitative separation of RNA polymerase I and II [3]. Fig. 2 shows the separation of RNA polymerase I and II from cells of *S. cerevisiae* Y185 grown at low and high specific growth rates (6.3 hr and 1.6 hr doubling time). Slow growing cells contain less RNA polymerase I than fast

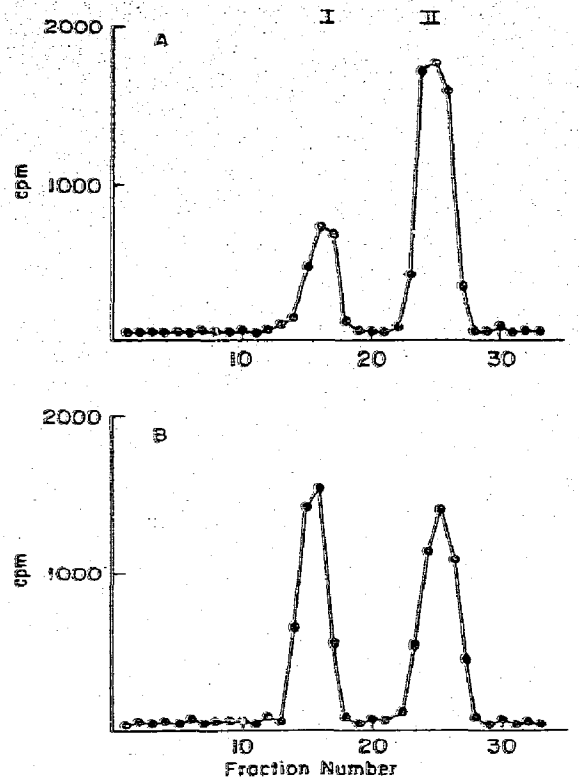


Fig. 2. Separation by DEAE sephadex chromatography of RNA polymerase I and II from extracts of *S. cerevisiae* Y185 grown at specific growth rates of 0.11 $hr^{-1}$ (A) and 0.43 $hr^{-1}$ (B). Samples of the yeast growing under steady-state conditions in the chemostat at growth rates $\mu = 0.11$ $hr^{-1}$ and $\mu = 0.43$ $hr^{-1}$ were harvested and cellular extracts were subject to chromatography on 20 ml DEAE Sephadex columns. The input of proteins was of 15 mg and the columns were eluted with the following stepwise gradient: 10 ml buffer B containing 0.05 M, 0.1 M, 0.2 M, 15 ml buffer B containing 0,25 M, 10 ml buffer B containing 0.3 M, 0.35 M and 0.45 M ammonium chloride. Fractions of 3 ml were collected from steps 0.05–0.25 and 0.35, 0.45 and 1.5 ml for steps 0.25 and 0.3. 50 µl of each fraction was assayed for RNA polymerase activity using poly dAT as template. 1000 cpm represent an incorporation of 8 pmol UMP into cold TCA precipitate material.

growing cells while the level of RNA polymerase II changes less with the change in the growth rate. No differences were found in the template specificity, metal optimum and α amanitin sensitivity of RNA polymerase I or II from slow and fast grown cells.

Fig. 3 shows the changes in specific activity of the RNA polymerases I and II in *S. cerevisiae* IL46 grow-

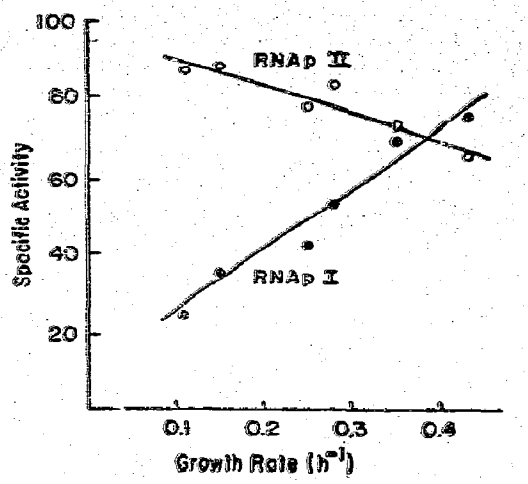


Fig. 3. Specific activity of RNA polymerase I (•) and II (○) in *S. cerevisiae* IL46 grown at different growth rates. The RNA polymerase I (•) and II (○) from cells grown at the growth rate indicated were separated by DEAE Sephadex chromatography and the activity of each of the enzymes was measured as indicated in Materials and methods and in the legend of fig. 2. The specific activity is given in units per mg protein.

ing at six different growth rates. RNA polymerase I decreases with the growth rate in a linear fashion showing a 3-fold difference between the slower and faster growth rates. RNA polymerase II is less affected by the growth rate although there is a slight but consistent increase in specific activity at low growth rates. It is evident from these results that there is a correlation between the specific growth rates, the RNA content of the cells and the level of RNA polymerase I. This relationship suggests that the synthesis of rRNA, the major component of the cellular RNA, can be regulated in fast and slow growing yeast cells by the level of RNA polymerases I activity. RNA polymerase I is believed to be the enzyme responsible for the synthesis of the rRNA in other eukaryotic organisms [9].

Fig. 4 shows the ratio of activity of RNA polymerase I and II at the various growth rates. The fact that the ratio of the two polymerases is variable at the different growth rates indicates that the yeast cells can regulate the concentration of the RNA polymerase I and II by independent control mechanisms.

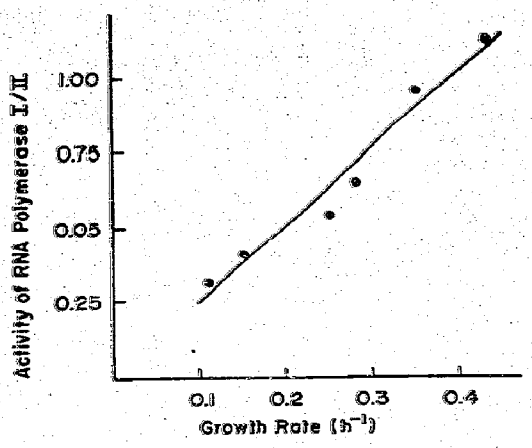


Fig. 4. Ratio of the activity of RNA polymerase I and II in *S. cerevisiae* IL46 grown at different growth rates.

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